Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo **Report of the In-house investigations of Glycerine in the developing chicken embryo** 5/3/74

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

DATE: May 3, 1974

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

THRU : Dr. Leo Friedman, Director
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M. Jacqueline Verrett

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Glycerine in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Glycerine

Protocol:

Glycerine (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Column 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Glycerine showed no toxicity when administered via the air cell at 0 hour between 25 and 1500 mg/kg. At 96 hours there was significant toxicity only at the highest dose administered, 500 mg/kg. In both cases the slope of the regression line was negative. Yolk treatment at 0 hours showed toxicity above solvent level between 50 and 1000 mg/kg, but the slope of the regression line was not significantly different from zero ($p=0.05$). At 96 hours yolk treatment also showed toxicity higher than background between 25 and 500 mg/kg, but the slope of the line was negative.

There were very few serious abnormalities observed for the four conditions of test. Using air cell treatment at 0 hours there were two serious anomalies at 1000 mg/kg, one exencephaly and one ablepharia; at 100 mg/kg there was a high incidence of abnormalities but it was predominantly due to minor abnormalities of the feet and ankles. Air cell treatment at 96 hours had one bird with ectromelia at 500 mg/kg, and one each with microphthalmia and a short mandible at 50 mg/kg. There was only one serious anomaly for yolk treatment at 0 hours, a bird with torticollis at 50 mg/kg. Yolk treatment at 96 hours had three birds with serious defects: 500 mg/kg, exencephaly; 125 mg/kg, celosomia; 50 mg/kg, buphthalmia; and the solvent controls had one abnormal bird that had dysgnathia and was blind. All untreated controls were normal.

Under the conditions of test glycerine showed slight toxicity only with yolk administration and showed no teratogenicity under any of the four conditions used.

1. Glycerine, Lot #DT07173P, Dow Chemical Co.

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006.

4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix 1.

Table 1

Glycerine

Air Cell at 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
75.0	1500.0	25	0.00	0.00	0.00
50.0	1000.0	105	13.33	4.76	4.76
25.0	500.0	55	21.81	3.63	0.00
12.50	250.0	105	17.14	0.95	0.00
6.250	125.0	25	24.00	0.00	0.00
5.00	100.0	80	20.00	11.25*	5.00*
2.50	50.0	105	13.33	0.95	0.95
1.250	25.0	65	32.30*	6.15*	3.07
Water		185	14.59	0.00	0.00
Controls		381	10.23	0.78	0.00

**Slope is negative

* Significantly different from solvent ($p \leq 0.05$)

Table 2

Glycerine

Air Cell at 96 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
25.0	500.0	100	71.00*	5.00	4.00
12.50	250.0	100	28.00	4.00	1.00
6.250	125.0	100	21.00	5.00	1.00
2.50	50.0	100	18.00	4.00	4.00
1.250	25.0	100	37.00	5.00	2.00
Water		105	28.57	0.95	0.00
Controls		381	10.23	0.78	0.00

**Slope is negative

* Significantly different from solvent ($p \leq 0.05$)

Table 3

Glycerine

Yolk at 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
50.0	1000.0	105	70.47*	3.80	1.90
25.0	500.0	105	85.71*	2.85	1.90
12.50	250.0	103	75.72*	3.88	0.00
5.00	100.0	104	63.46*	1.92	0.00
2.50	50.0	103	63.10*	7.76*	0.97
Water		160	18.75	1.25	0.62
Controls		381	10.23	0.78	0.00

**Slope not significantly different from zero (p=0.05)

* Significantly different from solvent ($p \leq 0.05$)

Table 4

Glycerine

Yolk at 96 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
25.0	500.0	100	69.00*	11.00*	3.00
12.50	250.0	100	48.00*	4.00	1.00
6.250	125.0	100	51.00*	8.00*	4.00
2.50	50.0	100	55.00*	6.00	4.00
1.250	25.0	100	54.00*	6.00	1.00
Water		110	29.09	0.90	0.90
Controls		381	10.23	0.78	0.00

**Slope is negative

* Significantly different from solvent ($p \leq 0.05$)